MECHANISM OF IS*1* TRANSPOSITION IN *E. COLI*: CHOICE BETWEEN SIMPLE INSERTION AND COINTEGRATION

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Manuscript received March 3, 1984
Revised copy accepted June 2, 1984

ABSTRACT

Insertion element IS*1* and IS*1*-based transposon Tn*9* generate cointegrates (containing vector and target DNAs joined by duplicate copies of IS*1* or Tn*9*) and simple insertions (containing IS*1* or Tn*9* detached from vector sequences). Based on studies of transposon Tn*5* we had proposed a conservative (nonreplicative) model for simple insertion. Others had proposed that all transposition is replicative, occurring in a rolling circle structure, and that the way DNA strands are joined when replication terminates determines whether a simple insertion or a cointegrate is formed.——We selected for the transposition of *amp* and *cam* resistance markers from pBR322::Tn*9* plasmids to an F factor in *recA*⁻ *E. coli* and identified products containing three and four copies of IS*1*, corresponding to true cointegrates (from monomeric plasmids), and simple insertions (from dimeric plasmids). The simple insertions with four copies of IS*1* outnumbered those with three by a ratio of about 3:1, whereas true cointegrates containing three copies of IS*1* were more numerous than those with four.——A straightforward rolling circle model had predicted that the simple insertions containing three copies of IS*1* should be more frequent than those with four. Because we obtained the opposite result we propose that simple insertions only arise when the element fails to replicate or if replication starts but then terminates prematurely. The two classes of products, simple insertions and cointegrates, reflect alternative conservative and replicative fates, respectively, of an early intermediate in transposition.

ANALYSES of transposable elements in prokaryotes have shown that their movement is independent of extensive DNA sequence homology and of the *rec* genes necessary for classical recombination. Transposition depends, instead, on element-specific proteins called transposases. These proteins may act by binding the ends of their cognate elements, introducing single- or double-strand breaks at the junctions of the element and its vector, cleaving the target DNA, joining element and target DNAs and, in certain cases, inducing the specific replication of the element (for reviews see IIDA, MEYER and ARBER 1983; BERG and BERG 1983; KLECKNER 1981).

Abbreviations used in text: *amp*, *cam* and *tet*, genes encoding resistances to ampicillin, chloramphenicol and tetracycline, respectively; *ori*, the origin of pBR322 replication.

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Two types of transposition products have been described, cointegrates and simple insertions. Cointegrates consist of complete vector and target DNAs joined by duplicate copies of the transposable element. These can arise in *recA*$^+$ (recombination-proficient) cells after the completion of transposition: a reciprocal (homologous) crossover between different DNA molecules, each with a copy of the element, will join them together in a recombinational cointegrate. Cointegrates can also arise without recombination when only one DNA molecule contains the transposable element; in this case their formation requires replication during transposition (SHAPIRO 1979; ARTHUR and SHERRATT 1979; GALAS and CHANDLER 1981; HARSHEY and BUKHARI 1981).

Simple insertions, in contrast, consist of the element moved to new genomic locations and free of vector sequences. Some arise secondarily from cointegrates by homologous recombination mediated by the *recA* protein or, in the case of transposon Tn*3* and related elements, by a site-specific reaction termed resolution (see HEFFRON 1983). However, simple insertions are also found as the primary products of transposition, and there are two views of how these arise. Based on our analyses of Tn*5*, which only gives simple insertions, we proposed a model of conservative transposition involving double-strand cleavages to separate the element from its vector and the splicing of the element into a cleaved target DNA molecule. In this model DNA synthesis is limited to the repair of gaps at the element-target junctions and leads only to the characteristic target sequence duplication [9 base pairs (bp) for Tn*5*], not to duplication of the element (BERG 1977, 1983). Other models have been built assuming that simple insertion, like cointegration, involves replication (GALAS and CHANDLER 1981; HARSHEY and BUKHARI 1981; HIRSCHEL, GALAS and CHANDLER 1982; HIRSCHEL *et al.* 1982; KLECKNER 1981).

The insertion element IS*1* (GALAS and CHANDLER 1982) and bacteriophage Mu (HARSHEY and BUKHARI 1981) are intriguing because they generate both cointegrates and simple insertions. To explain how IS*1* might give rise to both types of products GALAS and CHANDLER (1981) proposed that transposition is invariably linked to replication of the element in a rolling circle model (Figure 1); an equivalent model to explain the behavior of phage Mu was proposed independently by HARSHEY and BUKHARI (1981). In their models transposition is initiated when the transposase protein joins a strand at one end of the element to the target DNA. The fork that is generated promotes replication of element and vector sequences, and only when the wave of replication is terminated by the action of transposase at another recognition site is the choice between cointegration or simple insertion made.

We report here that among simple insertions from dimeric forms of pBR322::Tn*9* plasmids those with four copies of IS*1* outnumber those with three copies of IS*1*. This outcome suggests to us that simple insertions do not arise by a rolling circle mechanism. We propose, therefore, that IS*1* transposition can, but need not be, replicative. Both products may arise from a single intermediate: cointegrates whenever the element replicates and simple insertions if the element does not.

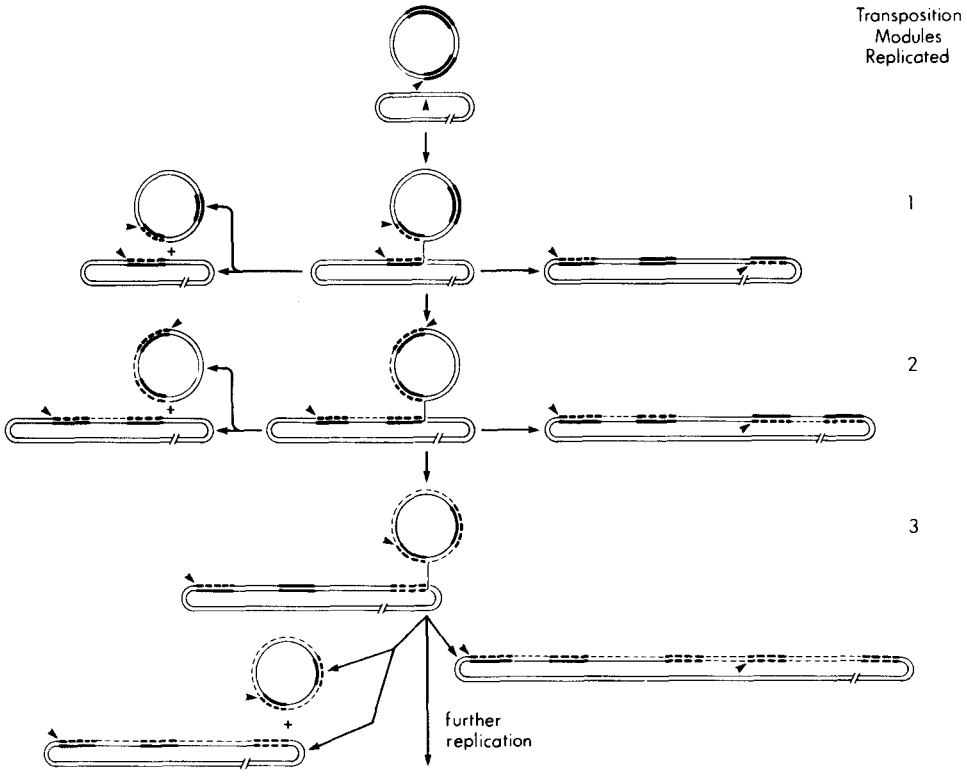

FIGURE 1.—Model of transposition associated with unidirectional (rolling circle) replication (adapted from HARSHEY and BUKHARI 1981; GALAS and CHANDLER 1981). Thick lines correspond to strands of the transposable element. The top circle represents a monomeric pBR322::Tn*9* plasmid in which direct repeats of IS*1* (thick lines) separate the *cam* gene (of Tn*9*) and the sequences of pBR322. Sequences replicated during transposition are indicated by dashed lines. Other models for transposition have been presented by BERG (1977, 1983), GRINDLEY and SHERRATT (1978), ARTHUR and SHERRATT (1979), SHAPIRO (1979) and OHTSUBO *et al.* (1980).

MATERIALS AND METHODS

Bacterial plasmids: The F factor pOX38 (GUYER *et al.* 1980) used as a target for transposition is about 52 kb long and contains the genes of F needed for autonomous replication and conjugal transfer but lacks the insertion elements present in wild-type F. The locations of restriction endonuclease cleavage sites in plasmid pBR322 (SUTCLIFFE 1978) and in transposon Tn*9* which contains direct terminal repeats of IS*1* (called IS*1*A and IS*1*B) and encodes resistance to chloramphenicol (Cam^r) (ALTON and VAPNEK 1979; OHTSUBO and OHTSUBO 1978; MACHIDA *et al.* 1982) are known. The pBR322::Tn*9* plasmids pBRG44 and pBRG45 were constructed by transposition of Tn*9* from an *E. coli* chromosomal site to a derivative of plasmid pBR322 mutated in its *Eco*RI site [by *Eco*RI cleavage, repair with DNA polymerase I (Klenow fragment) and blunt end ligation]. Our selection for transposition of Tn*9* to pBR322 has been described (BIEL, ADELT and BERG 1984). Restriction endonuclease mapping showed that in pBRG44 the Tn*9* element is bracketed by the *Sau*IIIA sites at pBR322 positions 3225 and 3330. It is oriented such that IS*1*A is closest to *amp* and IS*1*B is closest to *ori* (Figure 2). In pBRG45, Tn*9* is located in the promoter region of pBR322's *tet* gene just upstream from the *Hin*dIII site and oriented such that IS*1*A is

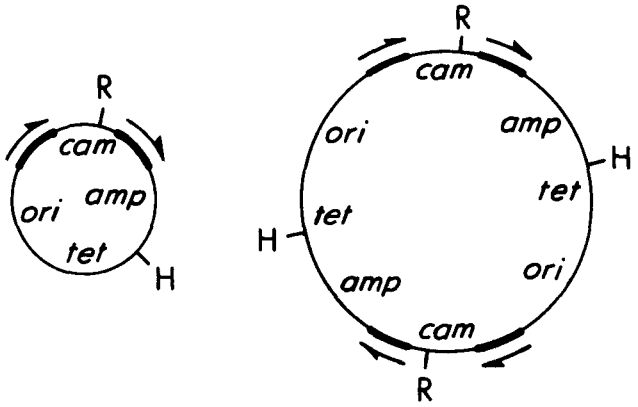

FIGURE 2.—Monomeric (left) and dimeric (right) forms of the pBR322::Tn*9* plasmid pBRG44. Abbreviations: *cam*, *amp* and *tet*, genes encoding resistances to chloramphenicol, ampicillin and tetracycline, respectively; *ori*, origin of replication; R and H, sites cleaved by restriction endonucleases *Eco*RI and *Hin*dIII, respectively. The *Eco*RI site normally present in pBR322 has been mutated so that the only site in pBRG44 cleaved by *Eco*RI is in the *cam* gene. The thick lines represent IS*1* sequences, and their orientations are indicated by arrows.

nearest the *Hin*dIII site. pBRG44 encodes resistance to chloramphenicol, ampicillin and tetracycline; pBRG45 encodes resistance to chloramphenicol and ampicillin but not to tetracycline because the Tn*9* insertion inactivated the *tet* gene promoter.

Dimeric forms of pBRG44 (Figure 2) and pBRG45 were generated by homologous recombination *in vivo* and maintained in *recA*$^-$ *E. coli* (see BERG 1983).

Bacterial strains: All bacterial strains were derived from *E. coli* K-12. DB1504 (BERG, WEISS and CROSSLAND 1980) is F$^-$ *recA*$^+$ Δ*trp*E5 Δ(*proB-lac*) and was used as the host to generate dimeric forms of pBR322::Tn*9* plasmids. DB1986 (BERG 1983) is Δ*trp*E5 Δ*rac* Δ(*srl*::Tn*10*-*recA*306) and harbors the F factor pOX38. Derivatives of DB1986 carrying pBR322::Tn*9* plasmids were used as donor strains in bacterial matings as described (BIEL, ADELT and BERG 1984). DB1648 (EGNER and BERG 1981) is F$^-$ *recA1* Δ*trp*E5 Δ(*proB-lac*) *rpsL* and was used as the recipient in conjugation.

General methods: The DNAs of small (pBR322 and pBR322::Tn*9*) and also of the larger (pOX38-pBR322::Tn*9*) chimeric plasmids were extracted by an alkaline-SDS lysis procedure (BIRNBOIM and DOLY 1979). Recipes for media, protocols for bacterial growth and matings and electrophoresis in agarose gels have been described (BERG 1983; BERG, SCHMANDT and LOWE 1983; BIEL, ADELT and BERG 1984; MANIATIS, FRITSCH and SAMBROOK 1982). Restriction endonucleases were used according to the suppliers' (New England Biolabs and Bethesda Research Laboratories) instructions.

RATIONALE

Definitions: Chimera is used as a general term to indicate any product of transposition between replicons. Simple insertions consist of a fragment of the donor molecule moved to a new location and separated from previously adjacent vector segments. By contrast, cointegrates consist of vector and target sequences joined by duplicate copies of the mobile element.

Experimental approach: The transposable element Tn*9* contains direct terminal repeats of the smaller mobile element IS*1* bracketing a region encoding chloramphenicol resistance (see Figure 2). The IS*1* elements encode the proteins and provide the sites necessary for Tn*9* movement, and probably any short DNA segment becomes transposable when bracketed by a pair of IS*1*

elements (MACHIDA *et al.* 1982). CHANDLER, CLERGET and GALAS (1982) studied the movement of IS*1*-based transposons from sites in a repressed prophage to a plasmid and found the probability of transposition to be a simple inverse function of DNA length. They interpreted this outcome as support for their rolling circle model, a reflection they believed of a gradual decay of a specialized replication fork. We have tested this interpretation using dimeric and monomeric forms of pBR322::Tn*9* plasmids (Figure 2).

Selection for the joint transposition of *cam* and *amp* determinants from dimeric pBR322:Tn*9* plasmids permits the recovery of several classes of simple insertions (Figure 3). One class contains three copies of IS*1* and single copies of the *cam* and *amp* segments; two other classes contain four copies of IS*1*, one with duplicate copies of the *cam* segment and a single copy of the *amp* segment, and the other with a single copy of the *cam* segment and duplicate copies of the *amp* segment. In each class only part of the dimeric donor plasmid had transposed and, thus, each is a simple insertion, not a cointegrate. According to the GALAS-CHANDLER rolling circle model, simple insertions containing three copies of IS*1* should outnumber those containing four copies of IS*1* because the replication fork need travel less distance to generate the former class.

pBR322::Tn*9* plasmids are nonconjugative, and transposition of their resistance genes to the F factor pOX38 can be selected in bacterial matings (BERG *et al.* 1980; GALAS and CHANDLER 1982). Derivatives of strain DB1986 harboring the pBR322::Tn*9* plasmids pBRG44 and pBRG45 were crossed with *recA*$^-$ recipient strain DB1648, and Cam^r Amp^r exconjugants were selected. Electrophoresis of plasmid DNA extracted from exconjugants generally revealed a single DNA species somewhat larger (about 8–20 kb) than pOX38 (GALAS and CHANDLER 1982; BIEL, ADELT and BERG 1984). However, a small fraction (about 15%) contained additional prominent bands of smaller DNAs, generally the size of pBR322::IS*1* or pBR322::Tn*9*. Our tests (BIEL, ADELT and BERG 1984) indicated that this heterogeneity reflects an instability of certain chimeras unrelated to the mechanism of transposition. The breakdown continued after transfer to new hosts, and cells harboring the unstable chimeras tended to make small colonies with fast growing sectors. We attributed this instability to a combination of factors including (1) rare *recA*-independent homologous recombination (which occurs even in plasmids that contain no IS elements; DOHERTY, MORRISON and KOLODNER 1983), (2) deleterious effects of certain insertions in pOX38 when maintained at high copy number and (3) growth rate selection of clones in which smaller plasmids had outcompeted these chimeras in replication (BIEL, ADELT and BERG 1984). Because unstable chimeras were infrequent, instability was apparently unrelated to transposition, and characterization of chimeric DNA structures is easiest with homogeneous plasmid preparations, the unstable chimeras were not studied further.

The structures and numbers of IS*1* elements present in stable pOX38-pBR322::Tn*9* can be inferred from the patterns of DNA fragments produced by digestion with appropriate restriction endonucleases. The pBR322::Tn*9* plasmids used here contain a single *Hin*dIII site in the pBR322 component

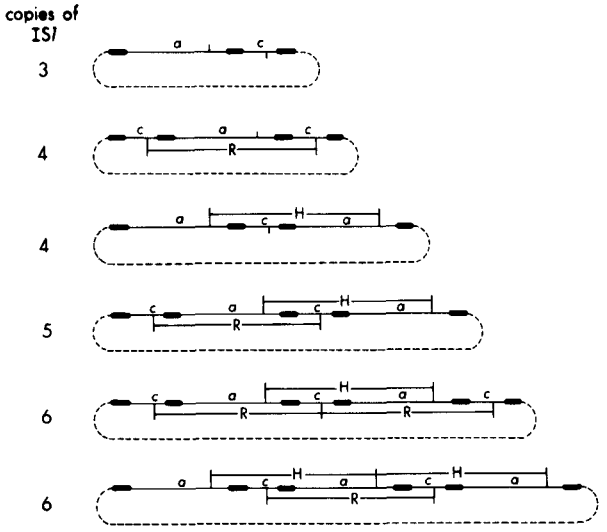

FIGURE 3.—Plan for restriction endonuclease analyses of the structures of pOX38-pBR322::Tn*9* chimeras. Dashed lines, pOX38 target; thick lines, IS*1* elements. a, *amp* gene (the pBR322 segment); c, *cam* gene (of Tn*9*); short vertical line in the *amp* segment, the *Hin*dIII site; short vertical line in the *cam* segment, the *Eco*RI site; the segments marked R and H correspond to the full length pBR322::Tn*9* segments generated by *Eco*RI and by *Hin*dIII digestion, respectively, of chimeras containing four or more copies of IS*1*. Chimeras containing three copies of IS*1* have only single *Eco*RI and *Hin*dIII sites in the transposed (pBR322::Tn*9*) segment. The single *Hin*dIII site and the numerous *Eco*RI sites in the pOX38 target DNA are omitted from the diagram since they are not important in our analysis (see Figure 4).

and a single *Eco*RI site in the *cam* gene (see Figure 2). Consequently, digestion of either plasmid with *Hin*dIII or with *Eco*RI generates a single linear fragment 7 kb long. As diagrammed in Figure 3 and illustrated in Figure 4, the absence of the 7-kb fragment from *Hin*dIII and from *Eco*RI digests indicates a chimera with just three copies of IS*1*. The presence of the 7-kb fragment in one digest but not in the other indicates a chimera with four copies of IS*1*. Finally, the presence of the 7-kb fragment in both digests indicates five or more copies of IS*1*.

RESULTS AND DISCUSSION

We analyzed the structures of simple insertions formed by the transposition of the *cam* and *amp* determinants of dimeric pBR322::Tn*9* plasmids to the F factor pOX38 in *recA*$^-$ *E. coli*. The data in Table 1 show that simple insertions containing four IS*1* elements outnumber those containing only three by about 3:1. In addition, among simple insertions with four IS*1* elements, those with two copies of the pBR322 segment were about eightfold more frequent than those with two copies of the *cam* segment (designated type II and type I, respectively, in Table 1).

Inherent in the rolling circle model of GALAS and CHANDLER (1981) and of HARSHEY and BUKHARI (1981) was a prediction that the smallest of the simple

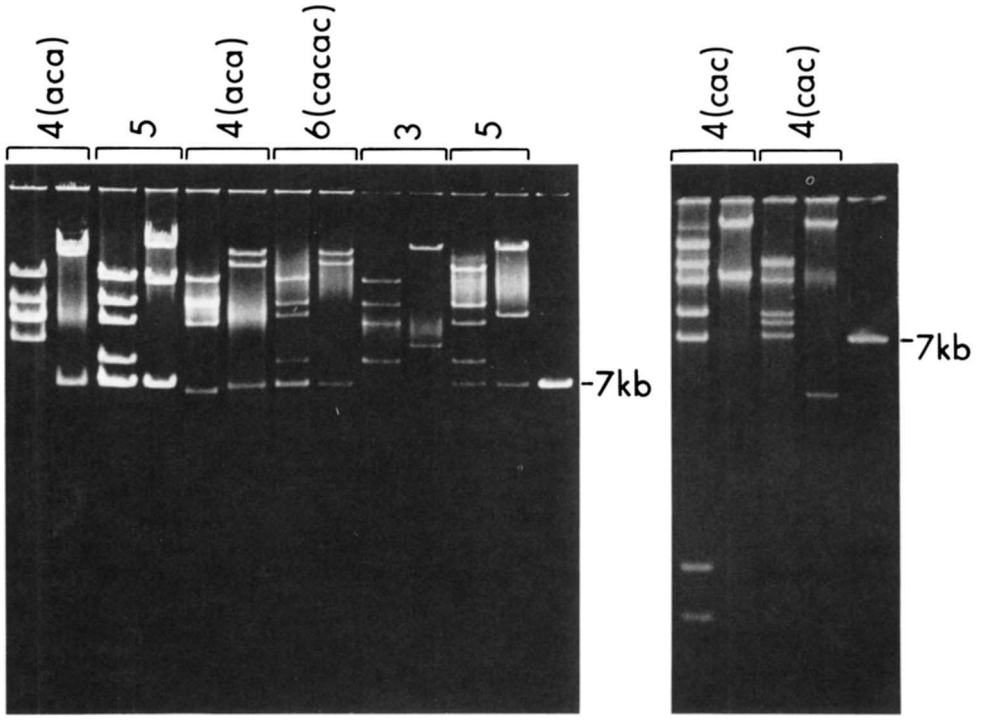

FIGURE 4.—Representative digests of pOX38-pBR322::Tn*9* chimeras. In each pair of lanes, the left contains the *Eco*RI digest, and the right contains the *Hin*dIII digest. The numbers 4, 5, 4, 6, etc. above each pair of lanes indicate the number of IS*1* elements that we interpret these chimeras to contain. The designations (aca), (cacac) and (cac) indicate the arrangement of pBR322 (a) and chloramphenicol resistance (c) segments deduced for these chimeras as shown in Figure 3.

insertions would be most frequent because their formation would have entailed the least DNA synthesis: expected to predominate was the class with three rather than four IS*1* elements and, among those with four IS*1* elements, the type with two copies of the *cam* segment and one of the *amp* segment (the 1102-bp *cam* segment is about one-fourth the length of the 4363-bp pBR322 plasmid). Our experiments (Table 1) gave just the opposite result.

Among true cointegrates (derived from monomeric pBR322::Tn*9* plasmids), those with three IS*1* elements were severalfold more frequent than those with four. This outcome is predicted by several models of transposition (including the rolling circle model) and thus serves as a valuable control. It is because the distribution of simple insertions from dimeric plasmids is opposite to that predicted by the rolling circle model of GALAS and CHANDLER (1981) and HARSHEY and BUKHARI (1981) that it became necessary to imagine how else transposition might occur.

Elements related to Tn*3* must move by a replicative process, since they invariably generate cointegrates (GILL *et al.* 1978; ARTHUR and SHERRATT 1979; HEFFRON 1983). However, the diversity evident in comparisons of DNA sequences, functional organization, regulation and transposition product struc-

TABLE 1

Size of DNA segments transposed

Donor	No. of IS*1* elements in product[a]					
	3	4 (Type I)[b]	4 (Type II)	5	>5	4 copies IS*1* / 3 copies IS*1*
Dimeric pBRG44	12	4	32	27	2	36/12
Dimeric pBRG45	12	5	45	7	1	50/12
Total	24	86		34	3	3.6
Monomeric pBRG44	72	11	9	3	1	20/72
Monomeric pBRG45	46	1	19	3	0	20/46
Total	118	40		6	1	0.34

[a] Each pOX38-pBR322::Tn*9* chimera was selected to contain both the *cam* and the *amp* determinants of the indicated pBR322::Tn*9* plasmid and was characterized by restriction endonuclease digestion (Figures 3 and 4). Each chimera is independent, having been derived from a mating of a separate donor subclone of strain DB1986 harboring the indicated pBR322::Tn*9* plasmid with recipient strain DB1648.

[b] Type I and type II indicate the arrangement of the segments encoding chloramphenicol resistance (of Tn*9*) and ampicillin resistance (of pBR322) in chimeras containing four copies of IS*1* (see Figure 3). Type I contains two copies of *cam* and one copy of *amp* (cac subclass), and type II contains two copies of *amp* and one copy of *cam* (aca subclass) (see Figure 3).

tures suggests that the current test of prokaryotic transposable elements may be derived from several different ancestral sources and that there may be more than one mechanism of transposition. Thus, we have suggested that transposon Tn*5*, unlike Tn*3*, may move by a conservative process since its transposition does not result in cointegrates (BERG 1977, 1983).

We propose that IS*1*-mediated transposition leading to simple insertion is conservative, whereas that leading to cointegrates is replicative (Figure 5). In our model, transposition is initiated by the binding of the transposase protein to both ends of the element, the cutting of one or both DNA strands at each of the element-vector junctions and the joining of the element's free ends to the cleaved target DNA. Single-strand breaks at the element-vector junctions lead to a pair of replication forks and to cointegrates. Double-strand breaks, on the other hand, separate vector and element sequences, do not permit the formation of replication forks and hence result in simple insertions. Thus, in our model, the structure of the transposition product is determined at the initiation step, not at termination.

The concentration of transposase may limit the rate of transposition, and transposase may be synthesized only sporadically from any single copy of IS*1*. Studies with mutated IS*1* elements had shown that the IS*1*-encoded transposase operates effectively only in *cis*, and that it is most active on the element that had encoded it (MACHIDA *et al.* 1982). This suggests that transposase may bind preferentially to an end of the element from which it was synthesized and then slide along the donor molecule in search of a second recognition site.

Figure 6 diagrams how sliding of transposase could account for the relative abundance of simple insertions containing four copies of IS*1* elements and also

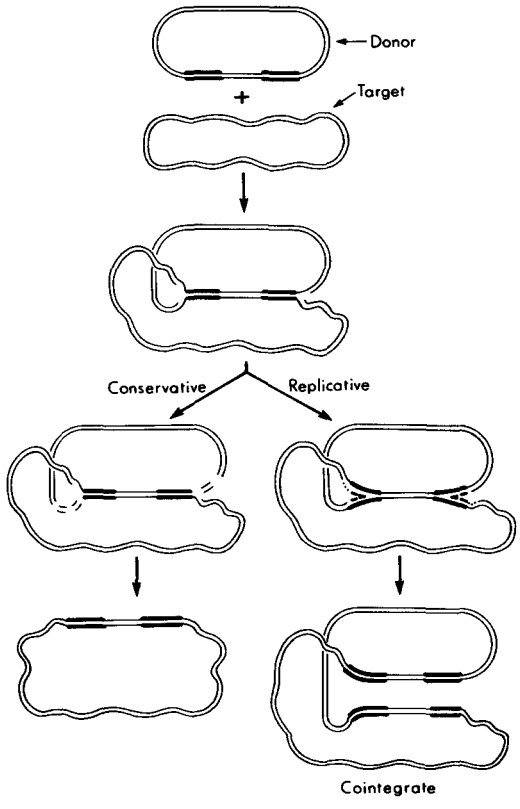

FIGURE 5.—Proposed formation of simple insertions and cointegrates from a single species of transposition intermediate. Represented here is transposon Tn*9* which contains direct terminal repeats of the insertion element IS*1* (thick lines). We propose that a key intermediate in transposition is generated by the joining of single strands at each end of the element to the target DNA. The choice between conservative (left branch) and replicative (right branch) fates depends on the relative probabilities of double- *vs.* single-strand breakage of the element vector-junctions prior to the onset of replication. Alternatively, breakage of arms of replication forks anytime before they meet would also generate simple insertions and would involve a limited amount of DNA synthesis instead of being completely conservative. The drawing depicts the transposition of the entire Tn*9* element. The transposition of a single IS*1* element would be initiated by breaks at its two ends.

for the excess of the *aca* subclass among simple insertions with four IS*1* elements (Table 1). Transposase synthesized from a single IS*1* element (arbitrarily, IS*1* number 1), binds to the "I" end of that element. It migrates until a second recognition site is bound, most frequently the I site of IS*1* number 4, less frequently the O end of IS*1* number 3, and results in simple insertions with four and three copies of IS*1*, respectively. Alternative binding to the O ends of IS*1* numbers 1 and 2 would generate the minority (*cac*) subclass of simple insertions containing four IS*1* elements.

Since true cointegrates from monomeric pBR322::Tn*9* containing three copies of IS*1* outnumber those with four copies of IS*1*, the *cac* subclass of cointegrates might, similarly, have been expected to outnumber the *aca* subclass since less DNA need be replicated. That the expected excess was not observed

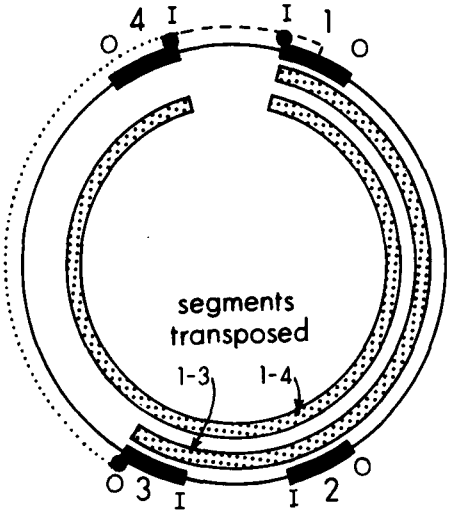

FIGURE 6.—Proposed tracking patterns of transposase synthesized sporadically from an IS*1* element. Solid boxes, IS*1* elements; filled circles, transposase; dashed and dotted lines, possible paths taken by transposase synthesized from a single IS*1* element; stippled boxes, segments transposed when transposase acts at the indicated ends of IS*1* elements numbers 1 and 3 or 1 and 4. O and I correspond to the outside and inside ends of IS*1* elements, respectively, as arranged in transposon Tn*9*. Even though the O and I ends of a single IS*1* element differ in DNA sequence, because IS*1* is repeated directly in Tn*9*, the O and I ends of adjacent IS*1* elements are identical in sequence. Even though the DNA is depicted here in extended form, we imagine that when transposase tracks in search of a second recognition site it may remain complexed with the first binding site. The two ends of the element would, accordingly, be brought together by the transposase protein complex (see BERG 1977).

(Table 1) also suggests to us fundamental differences between simple insertion and cointegration early in the transposition pathway. Transcription impinging on the end of an IS element decreases its ability to participate in transposition (SASAKAWA *et al.* 1982; MACHIDA *et al.* 1983; CHANDLER, CLERGET and GALAS 1982; BIEL, ADELT and BERG 1984). The unexpected abundance of the *aca* subclass among cointegrates may reflect this positional effect on those transposition events that are replicative.

We conclude that the striking inverse dependence of transposition frequency on DNA length during simple insertion events (CHANDLER, CLERGET and GALAS 1982; this work) may reflect only the probability that transposase bound to one recognition site will encounter a second site and thus be able to initiate transposition. It does not require that transposition occur by a replicative mechanism.

Because IS*1*, unlike Tn*5*, generates cointegrates as well as simple insertions, it seems possible that simple IS*1* insertion might also result from an initially replicative event interrupted by chance cleavages of the replication forks. This modification envisions a variable amount of DNA synthesis associated with such insertions, as has been found during the transposition of bacteriophage Mu *in vitro* (MIZUUCHI 1983). However, even this proposal, simple insertion due to an interruption of replication fork movement, differs fundamentally from roll-

ing circle models which require a full round of DNA synthesis during every successful transposition event and commitment to simple insertion or to cointegration only when the replication phase is complete.

We are grateful to G. ADELT for skillful technical assistance and to C. M. BERG for critical reading of this manuscript. This work was supported by United States Public Health Research grants 5 RO1 AI 14267 and 1 RO1 AI 18980 to D. E. B. S. W. B. was supported by Postdoctoral Training grant T32 AI-07172.

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Corresponding editor: G. MOSIG